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(FILE 'HOME' ENTERED AT 09:52:14 ON 03 JUN 2003)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 09:52:24 ON 03 JUN 2003

SEA CHITOBIASE

20 FILE AGRICOLA
1 FILE ANABSTR
45 FILE AQUASCI
11 FILE BIOBUSINESS
1 FILE BIOCOMMERCE
155 FILE BIOSIS
30 FILE BIOTECHABS
30 FILE BIOTECHDS
39 FILE BIOTECHNO
38 FILE CABA
213 FILE CAPLUS
6 FILE CEABA-VTB
5 FILE CONFSCI
7 FILE CROPU
37 FILE DDFB
23 FILE DGENE
37 FILE DRUGB
56 FILE EMBASE
49 FILE ESBIOBASE
2 FILE FEDRIP
2 FILE FROSTI
14 FILE FSTA
38 FILE GENBANK
8 FILE IFIPAT
17 FILE JICST-EPLUS
1 FILE KOSMET
64 FILE LIFESCI
55 FILE MEDLINE
3 FILE NTIS
23 FILE OCEAN
60 FILE PASCAL
1 FILE PHIN
1 FILE PROMT
133 FILE SCISEARCH
33 FILE TOXCENTER
51 FILE USPATFULL
3 FILE VETB
12 FILE WPIDS
12 FILE WPINDEX

L1

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FILE 'CAPLUS, BIOSIS, SCISEARCH, LIFESCI, PASCAL, EMBASE, MEDLINE, ESBIOBASE, AQUASCI, BIOTECHNO' ENTERED AT 09:54:09 ON 03 JUN 2003

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L3

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L3 ANSWER 1 OF 8 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:107593 CAPLUS
DOCUMENT NUMBER: 136:161320
TITLE: Use of ectoenzymes and secreted enzymes to monitor
cellular proliferation
INVENTOR(S): Zyskind, Judith
PATENT ASSIGNEE(S): Elitra Pharmaceuticals, Inc., USA
SOURCE: PCT Int. Appl., 80 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002010442	A1	20020207	WO 2000-US21049	20000802
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, VZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				

PRIORITY APPLN. INFO.: WO 2000-US21049 20000802

AB The invention concerns a method for measuring cellular proliferation in a sample comprising obtaining a sample of cells which express an ectoenzyme or a secreted enzyme, detg. the level of activity of the ectoenzyme or secreted enzyme in the sample and correlating the level of activity of the ectoenzyme or secreted enzyme with the extent of cellular proliferation. Further, secreted enzymes and ectoenzymes such as membrane-bound **chitobiase** (N,N'-diacetylchitobiase) and nucleic acids are used in combination with genetic methods to det. the impact of test compds. on cell proliferation.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:284144 CAPLUS
DOCUMENT NUMBER: 134:306156
TITLE: A cytoplasmic form of **chitobiase** as a **reporter enzyme** for the study of gene expression
INVENTOR(S): Zyskind, Judith
PATENT ASSIGNEE(S): Elitra Pharmaceuticals, Inc., USA
SOURCE: PCT Int. Appl., 44 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001027322	A2	20010419	WO 2000-US21048	20000802
WO 2001027322	A3	20011213		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, FI,				

GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
 RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.:

US 1999-159221P P 19991013

AB The present invention relates to reporter gene constructs encoding a cytoplasmic form of **chitobiase** (N,N'-diacetylchitobiase) and methods of using these reporter gene constructs. The use of a cytoplasmic form of **chitobiase** as a **reporter enzyme** is generally applicable to the study of gene expression in organisms which do not contain N-acetyl-.beta.-D-glucosaminidases.

L3 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2000:34968 CAPLUS

DOCUMENT NUMBER: 132:89234

TITLE: Fungal signal peptide and their use in secreting chitinolytic proteins from transgenic plants

INVENTOR(S): Harman, Gary E.; Lorito, Matteo; Woo, Sheridan; Brants, Aigars; Earle, Elizabeth; Kubicek, Christian P.; Peterbauer, Clemens K.; Tronsmo, Arne; Klemsdahl, Sonja

PATENT ASSIGNEE(S): Cornell Research Foundation, Inc., USA

SOURCE: PCT Int. Appl., 88 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000001812	A1	20000113	WO 1999-US15242	19990706
W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			

AU 9948621 A1 20000124 AU 1999-48621 19990706

PRIORITY APPLN. INFO.: US 1998-91768P P 19980706

WO 1999-US15242 W 19990706

AB Signal peptides from secreted proteins of fungi can be used to direct secretion of proteins from plant cells. Transgenic plants contg. expression constructs using these signal peptides can be regenerated. Specifically, signal peptides from chitinases of *Trichoderma harzianum* P1 are described. The chitinases can be used to improve plant resistance to insect pests and phytopathogenic fungi. An endochitinase and a **chitobiase** from cultures of *T. harzianum* were shown to inhibit fungal spore germination and to inhibit growth of insect larvae. A cDNA for the endochitinase was cloned by screening an expression library with antibodies to the enzyme. Similarly, a chitinase from *Gliocladium virens* was also found to have fungicidal effects. The *T. harzianum* chitinase cDNA was placed under control of a 35S **promoter** and introduced into tobacco and potato. Transgenic plants transcribed the gene and synthesized the protein with endogenous chitinase levels increased 10-400-fold. Transgenic plants showed greatly increased resistance to challenge with the airborne pathogen *Alternaria alternata* with some lines being completely resistant. Similarly, they also showed increased

resistance to the soil-borne *Rhizoctonia solani*.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 4 OF 8 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 1

ACCESSION NUMBER: 1999:288244 SCISEARCH

THE GENUINE ARTICLE: 184QP

TITLE: Multiple genes involved in chitin degradation from the marine bacterium *Pseudoalteromonas* sp. strain S91

AUTHOR: Techkarnjanaruk S; Goodman A E (Reprint)

CORPORATE SOURCE: UNIV S AUSTRALIA, SCH BIOL SCI, GPO BOX 2100, ADELAIDE, SA 5001, AUSTRALIA (Reprint); UNIV S AUSTRALIA, SCH BIOL SCI, ADELAIDE, SA 5001, AUSTRALIA

COUNTRY OF AUTHOR: AUSTRALIA

SOURCE: MICROBIOLOGY-UK, (APR 1999) Vol. 145, Part 4, pp. 925-934. Publisher: SOC GENERAL MICROBIOLOGY, MARLBOROUGH HOUSE, BASINGSTOKE RD, SPENCERS WOODS, READING RG7 1AE, BERKS, ENGLAND.

ISSN: 1350-0872.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 45

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A cluster of three closely linked chitinase genes organized in the order *chiA*, *chiB* and *chiC*, with the same transcriptional direction, and two unlinked genes, *chiP* and *chiB* involved in chitin degradation in *Pseudoalteromonas* sp. strain S91 were cloned, sequenced and characterized. The deduced amino acid sequences revealed that *ChiA*, *ChiB* and *ChiC* exhibited similarities to chitinases belonging to family 18 of the glycosyl hydrolases while *ChiP* and *ChiQ* belonged to family 20. *ChiP* and *ChiQ* showed different enzymic activities against fluorescent chitin analogues, but neither was able to degrade colloidal chitin. *ChiA* possessed chitinase activity but did not bind chitin; *ChiB* bound chitin but had no chitinase activity; *ChiC* possessed strong chitinase activity and also bound chitin. Production of *ChiC* in S91 appeared to be controlled by *chiA* expression, since insertion of a transposon into the ORF of *chiA* resulted in the loss of chitinase activity as well as loss of *ChiC* proteins in a chitinase-negative mutant. In *Escherichia coli*, *ChiC* appeared to be expressed from its own promoter.

L3 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 2

ACCESSION NUMBER: 1999:336322 CAPLUS

DOCUMENT NUMBER: 131:154270

TITLE: Structure of the human gene for lysosomal di-N-acetylchitobiase

AUTHOR(S): Liu, Bei; Ahmad, Wasim; Aronson, Nathan N., Jr.

CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, College of Medicine, University of South Alabama, Mobile, AL, 36688, USA

SOURCE: Glycobiology (1999), 9(6), 589-593

CODEN: GLYCE3; ISSN: 0959-6658

PUBLISHER: Oxford University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Chitobiase** is a lysosomal glycosidase that acts during the ordered degrdn. of asparagine-linked glycoproteins to cleave the core chitobiose unit at its reducing end. Human **chitobiase** is expressed in significant amts., while bovine **chitobiase** is produced at extremely low levels. To begin to understand this species-dependent expression, the authors detd. the gene structure of human **chitobiase**. The human **chitobiase** gene (CTBS) is approx. 20 kb comprising seven exons varying from 0.1 to 2.3 kb and six introns of 0.3 to 8 kb. The previously characterized partial bovine

chitobiase gene structure is similarly organized including exon and intron sizes and locations, but the human and bovine 5'-flanking regions differ significantly. 5'-RACE anal. of human **chitobiase** cDNA revealed only one transcriptional start site 45 bp upstream of the ATG translation initiation site. Computer anal. of the human **chitobiase** gene 5'-flanking region shows characteristics of a typical housekeeping gene. The putative **promoter** region contains a distal TATA box, and there are several Sp-1 and AP-2 cis elements. In contrast, bovine **chitobiase** gene 5'-flanking region shows totally different structures and may contain several silencers. A partial art-2 segment which is an artiodactyl Alu-like repetitive sequence, is also present. These evolutionary differences in the 5'-flanking region of the **chitobiase** genes from human and bovine could account for the widely varied expression levels of the hydrolase within these two species.

REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 3

ACCESSION NUMBER: 1999:3059 CAPLUS
DOCUMENT NUMBER: 130:149244
TITLE: **Chitobiase, a new reporter enzyme**

AUTHOR(S): Kalabat, D. Y.; Froelich, J. M.; Phuong, T. K.; Forsyth, R. A.; Newman, V. G.; Zyskind, J. W.
CORPORATE SOURCE: San Diego State University, San Diego, CA, USA
SOURCE: BioTechniques (1998), 25(6), 1030-1035
CODEN: BTNQDQ; ISSN: 0736-6205
PUBLISHER: Eaton Publishing Co.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB N,N'-diacetylchitobiase from the marine organism *Vibrio harveyi* is a highly stable **reporter enzyme** for gene fusions. This enzyme hydrolyzes the disaccharide chitobiose to N-acetylglucosamine. The advantages of the reporter gene encoding **chitobiase** (chb) are: (i) that **chitobiase** and N-acetyl-.beta.-D-glucosaminidase activities are missing in *E. coli* strains, (ii) **chitobiase** can be monitored using blue/white colony indicator plates and (iii) convenient substrates for this enzyme are com. available. The use of **chitobiase** as a **reporter enzyme** is generally applicable to the study of gene expression in those bacteria that do not contain N-acetyl-.beta.-D-glucosaminidases. The authors constructed plasmid vectors contg. a multiple cloning site for producing in-frame fusions to **chitobiase**, the attP of .lambda. phage for movement into the bacterial chromosome for single-copy anal., the gene encoding chloramphenicol acetyltransferase (cat), the pACYC184 origin of replication and the rrnBt1t2 terminator region upstream of the chb gene to prevent read-through from other **promoters**. In-frame fusions between the dnaA gene and chb were moved to the chromosome by site-specific recombination with the chromosomal attB site. These single-copy fusions were assayed for **chitobiase** to examine the effects of a deletion in the dnaA regulatory region.

REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 4

ACCESSION NUMBER: 1990:231809 CAPLUS
DOCUMENT NUMBER: 112:231809
TITLE: N,N'-Diacetylchitobiase of *Vibrio harveyi*. Primary structure, processing, and evolutionary relationships
AUTHOR(S): Soto-Gil, Rafael W.; Zyskind, Judith W.
CORPORATE SOURCE: Mol. Biol. Inst., San Diego State Univ., San Diego, CA, 92182, USA
SOURCE: Journal of Biological Chemistry (1989), 264(25),

14778-83

CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE:

Journal

LANGUAGE:

English

AB The nucleotide sequence of the gene *chb*, encoding the outer membrane protein, N,N'-diacetylchitinase (**chitinase**), of the marine bacterium, *Vibrio harveyi*, was detd. The amino acid sequence of prechitinase was derived from the nucleotide sequence. Prechitinase has a mol. mass of 97,771 Da and consists of 883 amino acid residues. A characteristic signal peptide is at the N-terminus whose removal is inhibited by the antibiotic, globomycin, suggesting that mature **chitinase** is a lipoprotein with a maturation pathway similar to that of the *Escherichia coli* major outer membrane lipoprotein. A perfect homol. of 6 amino acids at the processing and modification region of the outer membrane lipoprotein of *E. coli* was found with amino acids 15-19 of the deduced prechitinase protein sequence. **Chitinase** shares similarities and possibly common ancestry with the .alpha.-chain of the human .beta.-hexosaminidase. A comparison of the amino acid sequences of **chitinase** and the .alpha.-chain of .beta.-hexosaminidase gave a highly significant alignment score of 19.1 std. deviation units above a mean randomized alignment score. Primer extension anal. of the **promoter** region revealed 3 transcription initiation sites used by *E. coli* cells harboring the *chb* gene, 2 of which were also evident in *V. harveyi* cells.

L3 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2003 ACS

DUPLICATE 5

ACCESSION NUMBER: 1986:492435 CAPLUS

DOCUMENT NUMBER: 105:92435

TITLE: Chitinase determinants of *Vibrio vulnificus*: gene cloning and applications of a chitinase probe

AUTHOR(S): Wortman, A. T.; Somerville, C. C.; Colwell, R. R.

CORPORATE SOURCE: Dep. Microbiol., Univ. Maryland, College Park, MD, 20742, USA

SOURCE: Applied and Environmental Microbiology (1986), 52(1), 142-5

CODEN: AEMIDF; ISSN: 0099-2240

DOCUMENT TYPE:

Journal

LANGUAGE:

English

AB To initiate study of the genetic control of chitinolytic activity in vibrios, the **chitinase** [9012-33-3] gene was isolated by cloning chromosomal DNA prepd. from *V. vulnificus*. Chimeric plasmids were constructed from *Sau*3A I partial digests of chromosomal DNA prepd. by ligating 5-15-kilobase fragments into the *Bam*HI site, i.e., in the tetracycline-resistance (*Tcr*) gene, of pBR322 (*AmrTcr*). The resulting plasmids were transformed into *Escherichia coli* DH1. **Chitinase** activity of the insert-bearing clones was detected by using a chromogenic substrate, p-nitrophenyl-N-acetyl-.beta.,D-glucosaminide, and confirmed by the appearance of a fluorescent end product from the hydrolysis of 4-methylumbelliferyl-.beta.,D-N-N'-diacetylchitobiose. Endochitinase activity was demonstrated by liberation of water-sol. products produced by the degrading of [³H]chitin. Transformation of *E. coli* Y10R (*lacY*) with plasmids from chitinase-pos. clones restored the lactose-pos. phenotype, suggesting the presence of a permease assocd. with chitinase activity. Phys. mapping of plasmids contg. the chitinase determinants indicate that transcription of these genes in *E. coli* may be initiated at a *V. vulnificus* **promoter**.